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STUDIES ON THE INHALATION TOXICITY OF DYES PRESENT IN COLORED SMOKE MUNITIONS
FINAL REPORT FOR PHASE I STUDIES: GENERATION AND CHARACTERIZATION OF DYE AEROSOL

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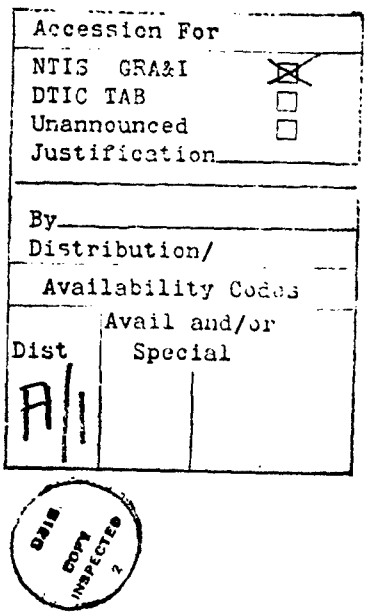

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Exposure Atmosphere

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The USAMBRDL has an interest in the potential inhalation toxicity of yellow dye (SY) and a yellow/green dye mixture (SY/SG) used in colored smoke munitions. A method for generation of respirable size particles of SY and SY/SG dye materials for inhalation toxicity studies with these dyes has been developed.

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EXECUTIVE SUMMARY

The USAMBRDL has an interest in the potential inhalation toxicity of yellow dye (SY) and a yellow/green dye mixture (SY/SG) used in colored smoke munitions. The chemical name of the major component of the yellow dye is 2-(2'quinolyl)-1,3-indandione (QI) and the major component of the green dye is 1,4-di-p-toluidinoanthraquinone (TA). To test the inhalation toxicity of these materials, one must be able to generate an exposure atmosphere of the material in a particle size in the respirable range. This is a report of the development of a methods to generate an exposure atmosphere of SY and SY/SG dyes and of the physical and chemical characterization of that atmosphere.

The stock dyes consisted of elongated particles with a length to diameter ratio of approximately 3. The count median diameter (CMD) of the SY dye was 2.0 μm with a geometric standard deviation (σ_g) of 2.0. The CMD of the SY/SG dye was 3.1 μm with a σ_g = 2.2. Both dye materials were sticky and particles tended to agglomerate into large particles. Generation of aerosols of the dyes required a method that would deagglomerate the clumps of particles to produce a respirable size aerosol. Chemically, the dye materials were 93-95% pure with the major contaminants being the precursors used in synthesis of the QI and TA or in the case of the yellow dye, an apparent artifact of the synthesis process in which 3 instead of 2 molecules have combined.

The use of a fluidized bed generator to produce aerosols of the dye materials was rejected because of the tendency of this type of generator to cause agglomeration of the dye particles. The generator of choice was one which uses high pressure air jets to deagglomerate and disperse the dye material into the exposure chambers. This generator is a commercial unit called a Jet-O-Mizer (Fluid Energy Crop., Hatfield, PA). Using this generation method with a single-stage impactor in the line between the generator and the exposure chamber (a 2-m^3 Hazleton 2000 chamber), aerosol atmospheres ranging from approximately 20 to 250 mg/m^3 with particles of a mass median aerodynamic diameter (MMAD) from 3.0 to 5.4 μm and a σ_g of approximately 2.0 were achieved. This represented a polydisperse aerosol of the size range desired for inhalation toxicity studies. The aerosolized dye materials did not change in chemical composition upon aerosolization.

A chamber homogeneity study was done in which the ratio of the concentration of aerosol at any of various points within the exposure chamber to the concentration at a central reference point was determined. Some statistically significant inhomogeneities were observed but should have minimal effects on the lung burdens of animals exposed in the chamber. The range in the mean ratios for each type of sampling position in the chamber (back, center, front; top, middle bottom; right side, left side) was from 0.92 to 1.10. (A ratio of 1.0 indicates no difference in concentration between the central reference point and the other sampling position in the chamber). Animal variation in amount of deposition of the particles in the respiratory tract can be expected to be greater than the observed inhomogeneities in distribution of aerosol in the chamber.

In summary, a method for generation of respirable size particles of SY and SY/SG dye materials for inhalation toxicity studies with these dyes has been developed.

ACKNOWLEDGMENTS

The authors acknowledge the outstanding contributions of all members of the Inhalation Toxicology Research Institute, without whose help these studies could not have been completed. Research supported by the U. S. Army Medical Bioengineering Research and Development Lab under a Memorandum of Understanding Agreement No. AT(29-2)-2138/3807 with the Lovelace Inhalation Toxicology Research Institute, which is operated for the U. S. Department of Energy under DOE Contract No. DE-AC04--76EV01013.

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INTRODUCTION

The Health Effects Research Division of the U. S. Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) needs to obtain information on the inhalation toxicity of the dyes present in colored smoke munitions. The major concern is for the health of munition production workers who could be exposed to fine dusts containing the dyes during mixing of the dry components of the smoke munition. In the project, "Studies on the Inhalation Toxicity of Dyes Present in Colored Smoke Munitions," the Lovelace Inhalation Toxicology Research Institute (ITRI) is studying the inhalation toxicity of two dye materials: a yellow dye (SY) and a green/yellow dye mix (SG/SY). The chemical name of the yellow dye is 2-(2'-quinolyl)-1,3-indandione (QI). Various synonyms used for the dye include C.I. solvent yellow 33, C.I. no 47000 and D & C yellow No. 11. The green dye is 1,4-di-p-toluidinoanthraquinone (TA) and has been called C.I. solvent green 3, C.I. 61565 and D & C Green No. 6. The green/yellow dye mix contains approximately 30 percent yellow dye and 70 percent green dye. The smoke munitions will contain 42 percent by weight of the dyes. The munition also contains potassium chlorate, magnesium carbonate and sucrose.

The work is being conducted in four phases. Phase I includes standardization of methods for generation of aerosols of the test materials and physical/chemical characterization of the aerosols. Phase II consists of range-finding experiments to determine acute toxic effects from exposure to high concentrations of the dyes and to select exposure concentrations for the next two phases of the study. In Phase III, one-month exposures of animals to varying concentrations of the dyes will be used to determine the lowest exposure concentration that will produce pathological changes. Phase IV will be a 90-day subchronic study to determine a no-observable-adverse-effects level (NOAEL) of exposure.

This is a final report of the work completed in Phase I of these studies.

PHYSICAL CHARACTERIZATION OF DYES

The size distribution and shape of both SY and SG/SY particles from stock material were determined using the transmission electron microscope (TEM). The stock materials were dispersed in water, and one drop of the suspension was put on an electron microscopic grid. The grid was then examined under a Zeiss EM 109 microscope. The size distribution of the projected area diameter and the aspect ratio (the length-to-diameter ratio of the elongated particle) were determined. Figures 1-3 show that both dye materials are elongated particles with aspect ratios of about 3. Table 1 lists both size distribution and the aspect ratio for the stock SY and SG/SY dyes.

Figure 1. Transmission electron micrograph of SY dye prepared from stock material.

Figures 2 & 3. Transmission electron micrograph SY/SG dye prepared from stock material

Figure 2

Figure 3

Table 1. Physical Characteristics of Dyes

Material	Aspect Ratio	Project Area Diameter (μm)		
	Mean ± S.D.	CMD	σ_g	Calculated MMAD[a]
Stock SY/SG	2.8 ± 1.5	3.1	2.2	20
Stock SY	3.1 ± 2.3	2.0	2.0	8.4
SY Aerosol	3.1 ± 1.5	0.8	1.9	2.8

a. MMAD = Mass Median Aerodynamic Diameter. These are estimated values based on a formula for spherical particles. They may not be accurate for elongated particles such as the stock dyes.

We also sampled airborne particles of SY dyes produced during aerosolization of the dye by a Jet-O-Mizer generation system (Figure 4). The samples were collected inside the exposure chamber using a point-to-plane electrostatic precipitator (ESP). These samples were taken during test runs designed to evaluate the generator. Size distributions and aspect ratios are also listed in Table 1. The size of the airborne particles was smaller than the stock material because larger particles were removed from the aerosol stream by sedimentation and by a one-stage impactor in the delivery system (Figure 5). However, the aspect ratio remained about 3. No airborne SG/SY particles were collected because of sparking by the ESP during the collection process.

CHEMICAL CHARACTERIZATION OF DYES

RECEIPT AND STORAGE OF DYES

The yellow and the green/yellow dye mixture were received from USAMBRDL on January 25, 1983. Each dye (the yellow and the green-yellow mix) was received packaged in a large plastic bag. The two bags had been shipped in a single cylindrical shipping carton. A total of 22.8 kg of green/yellow dye mixture and 50.2 kg of yellow dye was received. On March 9, 1983, each dye was divided into portions of about 1.5 kg, placed in polypropylene (Nalgene) bottles, and stored in the dark at room temperature.

To conduct valid inhalation toxicology studies on the dyes, analytical methods were needed to determine purity, stability during storage and aerosol generation, and concentrations in exposure atmospheres.

QUANTITATION OF MAJOR COMPONENTS OF THE DYES

Methods were developed to quantitate the major component of the yellow dye (2-(2'-quinolyl)-1,3-indanone; QI) and of the yellow/green dye mixture (1,4-di-p-toluidinoanthraquinone; TA) (Figure 6). These assays were developed to monitor stability of the dyes during storage and aerosol generation, and to quantitate the amount of dye in the exposure atmospheres.

Preparation of Standards

Analytical standards of QI and TA were prepared from the dyes supplied by USAMBRDL. Yellow dye was recrystallized twice from ethyl acetate, giving pure QI as analyzed by high-pressure liquid chromatography (HPLC) (Figure 7). The mass spectra (direct exposure probe, electron impact ionization) of this material (Figure 8) gave a molecular ion at 273, the molecular weight of QI. No evidence of the 6-methyl derivative of QI was found by HPLC or mass spectral analysis of the yellow dye. Likewise, the yellow/green dye mix was recrystallized three times from ethyl acetate, giving pure TA as determined by HPLC (Figure 7). Mass spectral analysis (Figure 9) indicated a molecular weight of 418, which corresponded to that of TA. No QI was detected.

Figure 4. Aerosolized SY dye collected from an exposure chamber.

Figure 5. Exposure system using a Jet-O-Mizer aerosol generator.

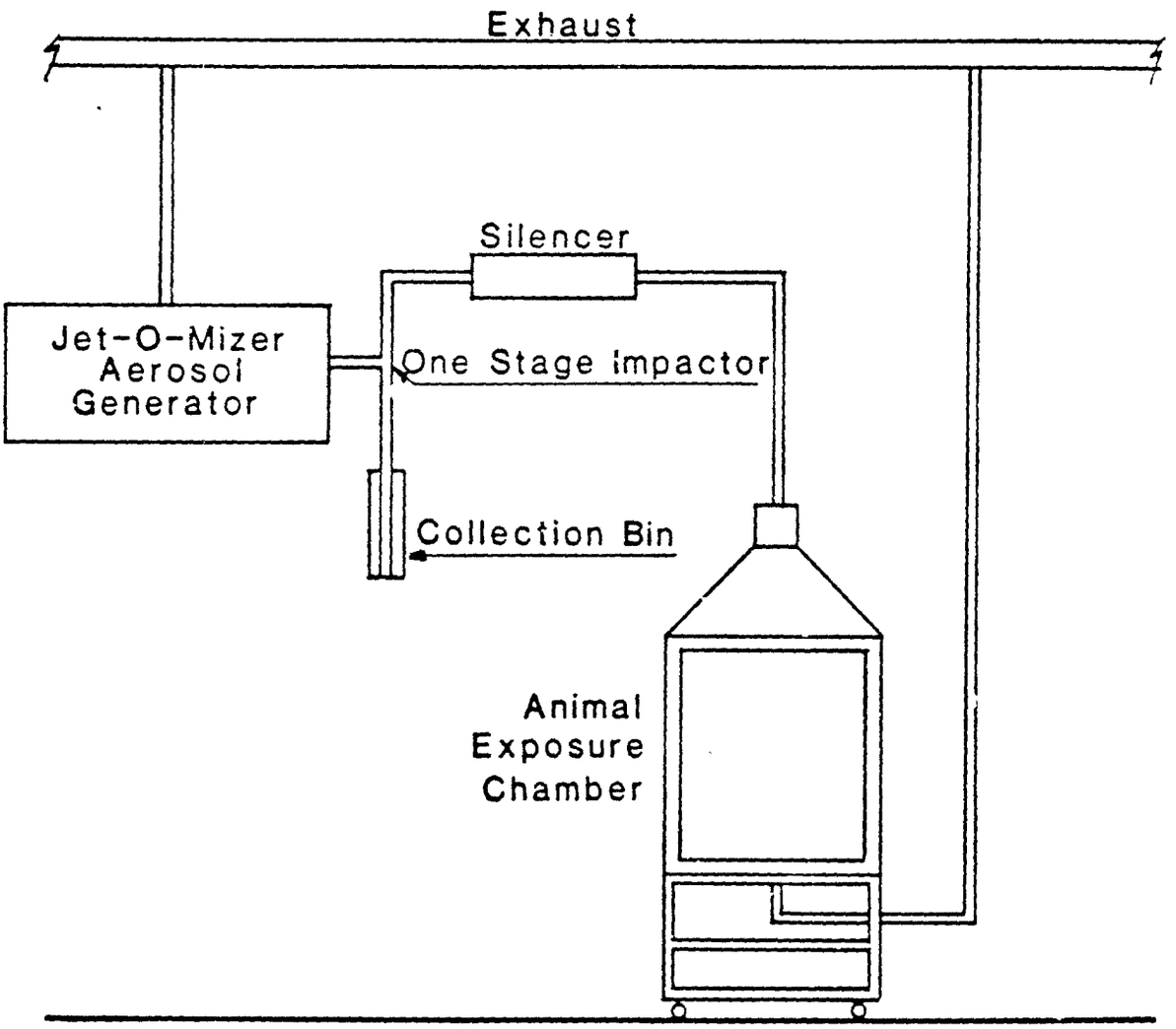
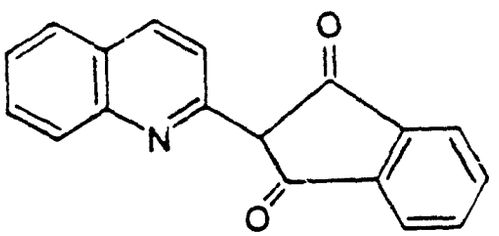
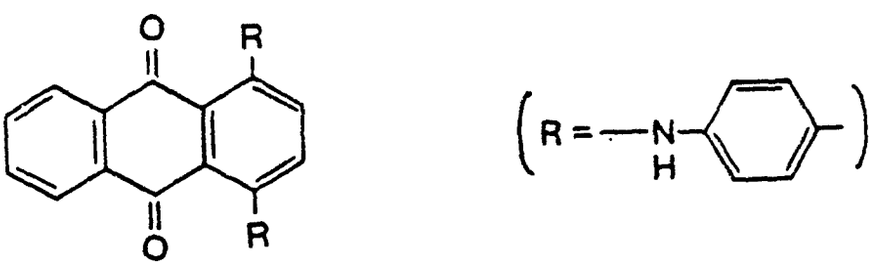


Figure 6. Chemical structures of the major chemical components of the yellow (QI) and the yellow/green dye mixture (QI and TA).



2-(2′-quinolyl)-1,3-indandione (QI)



1,4-di-p-toluidinoanthraquinone (TA)

Figure 7. Purity of QI and TA internal standards demonstrated by high performance liquid chromatography. Reverse phase column; methanol mobile phase for TA, 85:15 methanol: water mobile phase for QI; detector set at 254 nm.

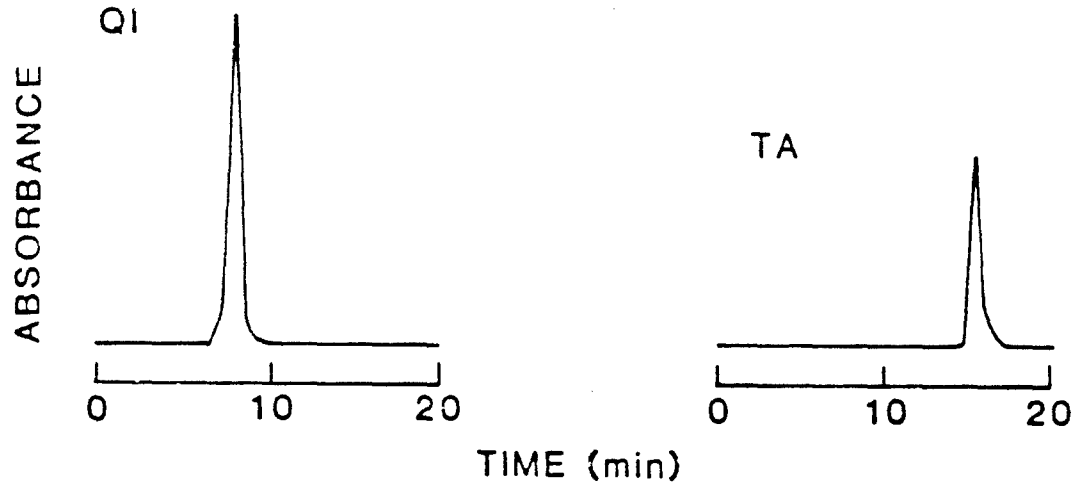


Figure 8. Mass spectrum of 2-(2'-quinolyl)-1,3-indandione, the major component of solvent yellow dye.

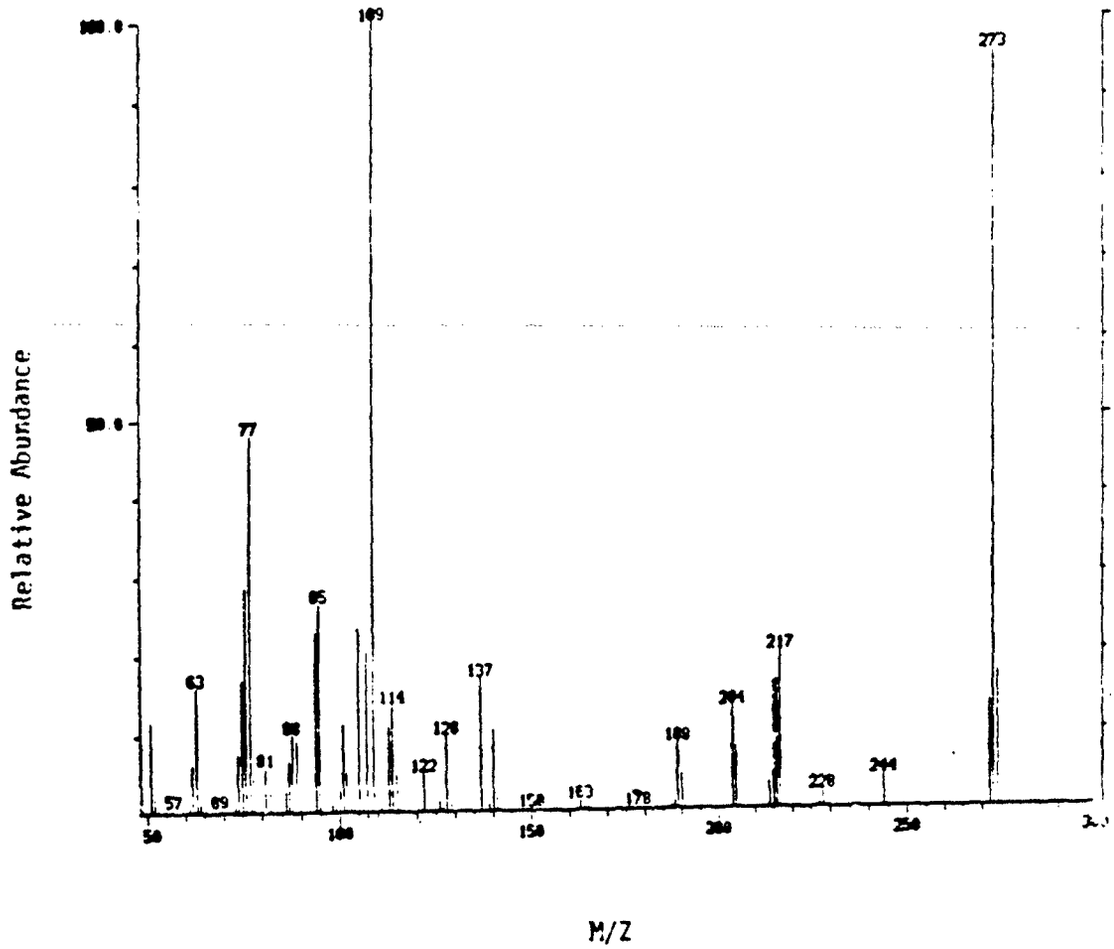
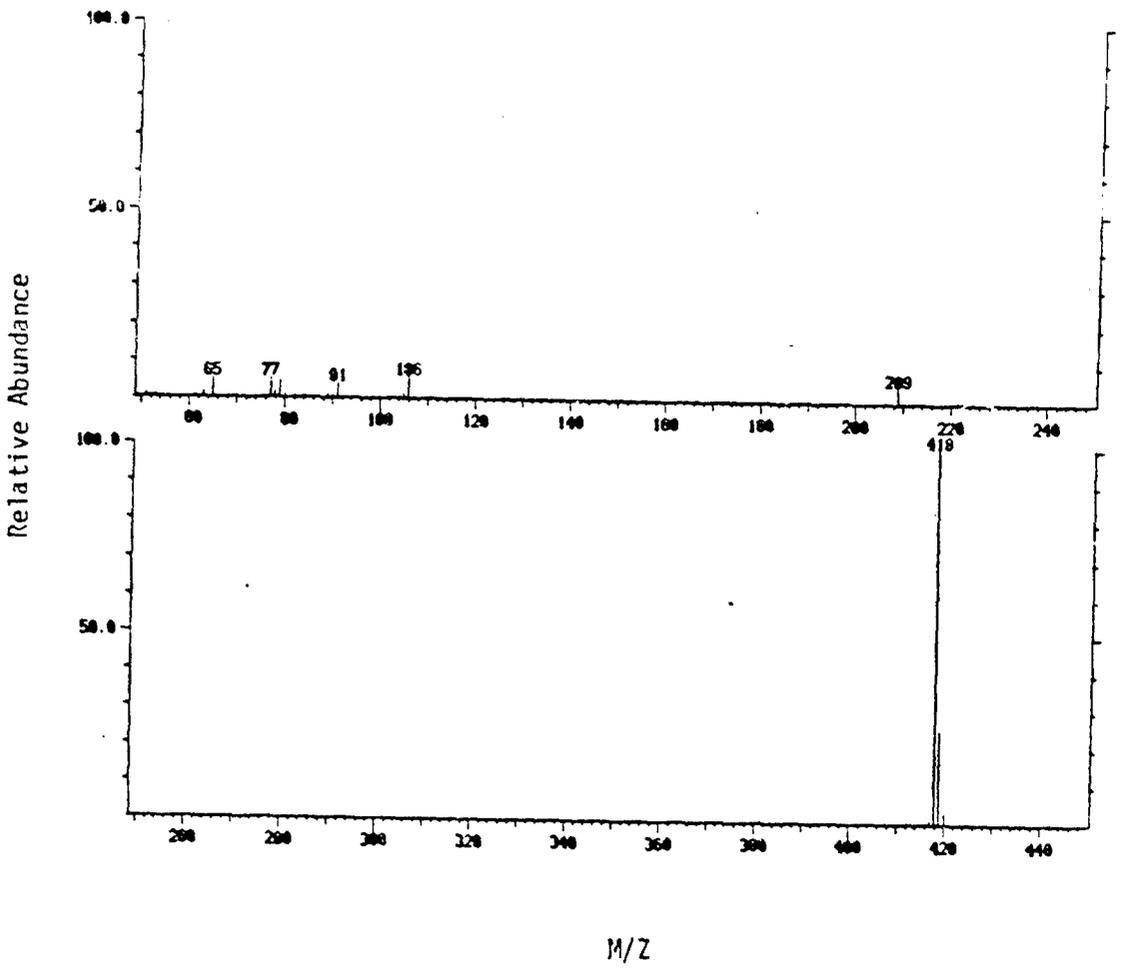


Figure 9. Mass spectrum of 1,4-di-p-toluidinoanthraquinone, the major component of solvent green dye.



Analyses of QI and TA

The HPLC conditions used to determine the amount of QI and TA in the dye samples were:

Altech RP 600 C-18, 4.1 mm x 25 cm column, 10 μm particle size
90:10 Methanol:water to 100 percent methanol in 10 min
100 percent methanol for 18 min
1 mL/min flow rate
UV detection @ 254 nm

QI and TA standards were dissolved in methanol and analyzed, giving the standard curve shown in Figure 10. Samples from five different bottles of yellow dye and four different bottles of green dye were dissolved in methanol and analyzed by HPLC. The results are shown in Table 2. Both dyes were found to be over 93 percent pure, and the ratio of TA to QI in the green/yellow dye was 2.9 ± 0.1 (mean ± SD).

Analysis of Filter Samples

Filter samples of aerosolized yellow dye were collected and extracted three times with tetrahydrofuran. Previous studies demonstrated that three extractions removed approximately 100 percent of the dye. The tetrahydrofuran was evaporated, and the residue was dissolved in methanol and analyzed for QI by HPLC. The amount of QI detected divided by the weight of dye on the filters is shown in Table 3. Filter samples of aerosolized yellow/green dye were extracted four times with tetrahydrofuran and analyzed for both QI and TA in a similar fashion. Weight of QI plus TA compared to the weight of dye on the filters is also shown in Table 3. These studies demonstrated that no significant decomposition of either dye took place during aerosolization.

Distribution of TA and QI vs Particle Size

HPLC analysis of impactor samples of aerosolized yellow/green dye was performed to determine if all particle sizes contained the same ratio of QI to TA. The results of analysis of one set of cascade impactor stages taken during a 1-hour generation of an aerosol of the yellow/green dye mixture are shown in Table 4. It was found that the QI and TA were uniformly distributed throughout all particle sizes except for those > 11 μm. Because this stage contained only 0.9 percent of the total mass of dye collected, it was judged to be of little significance.

Quantitation of Trace Impurities in Dye Samples

Several samples of yellow and yellow/green dye were analyzed for potential impurities from the manufacturing process. These impurities were phthalic acid, phthalic anhydride, and quinaldine for the yellow dye and these plus quinazarin and p-toluidine for the yellow/green dye mixture.

Figure 10. Standard curves for QI and TA quantitation in dye samples. Data are means ± SD, n = 10.

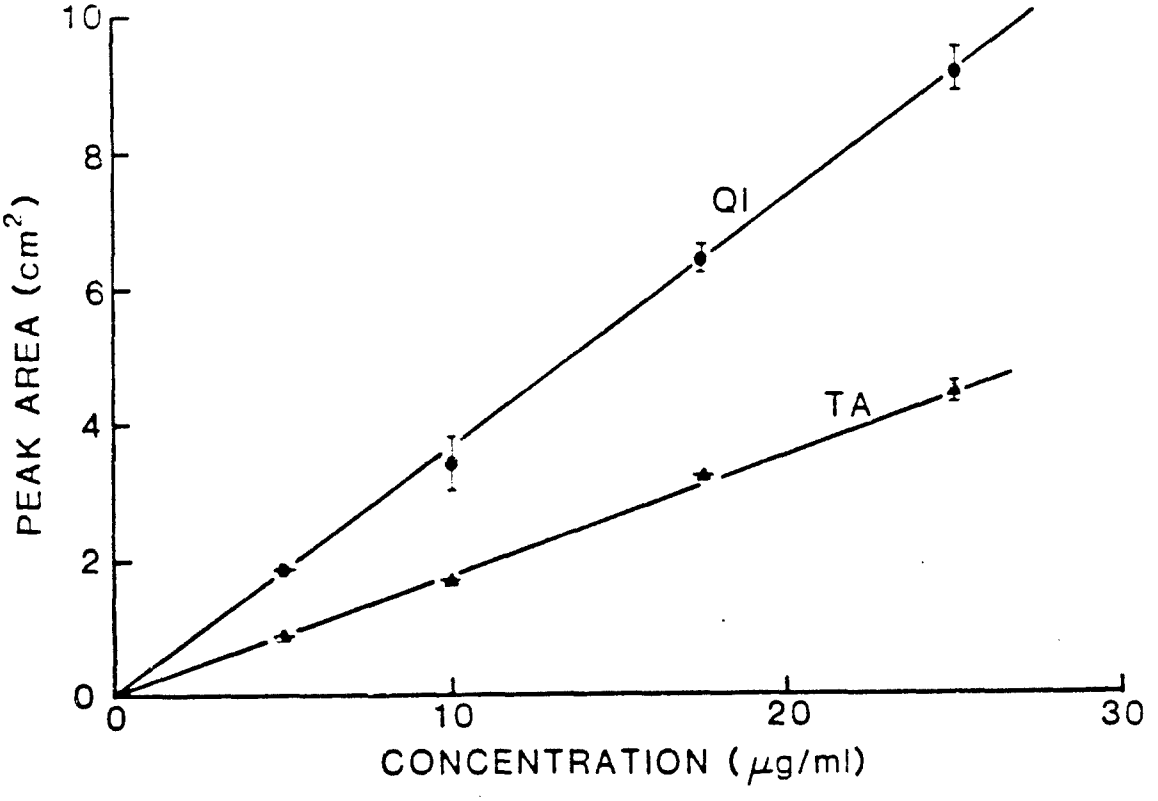


Table 2. Quantitation of QI and TA in Dye Samples

Dye	Weight % (mean ± SD)		
	QI	TA	Total
Yellow[a]	93.1 ± 1.3	-	93.1 ± 1.3
Yellow/green mix[b]	24.1 ± 0.5	70.9 ± 1.1	95.0 ± 1.0

a. n = 5

b. n = 4

Table 3. Quantitation of QI and TA in Dye Samples after Aerosolization

Dye	Weight % (mean ± SD)		
	QI	TA	Total
Yellow[a]	92.0 ± 2.7	-	92.0 ± 2.7
Yellow/green mix[b]	25.7 ± 1.6	71.9 ± 2.7	97.7 ± 4.2

a. n = 12

b. n = 3

Table 4. Yellow/Green Dye Composition of Different Size Aerosol Particles

Impactor Stage	Cut-off Diameter (μm)	QI/TA Ratio[a] (wt/wt)
1	11.4	0.69
2	7.3	0.30
3	4.7	0.30
4	2.9	0.35
5	1.9	0.30
6	1.13	0.29
7	0.7	0.30
8	< 0.7	0.30

a. QI = major component of yellow dye

TA = major component of green dye

For yellow dye, the same HPLC conditions described for quantitation of QI (described earlier) were used. The HPLC conditions used to analyze the yellow/green dye mixture were:

Altech RP C18, 4.1 mm x 25 cm column, 10 μm particle size
1 mL/min flow rate
UV detection @ 254 mm
Acetonitrile: acetate buffer (pH = 2.3; 3.9M)
60 to 70 percent acetonitrile in 10 min, hold at 70 percent for 5 min, 70 percent to 100 percent acetonitrile in 20 min

Figures 11 and 12 show typical chart tracings for injection of 1 μg of yellow dye and yellow/green dye mix, respectively. Known amounts of suspected impurities were injected to establish retention times (marked on chart tracing) and standard curves to quantitate the amount of each impurity. The area of unknown peaks in the chart tracing was compared to the area of the QI or the QI plus the TA peak. The results of this analysis are given in Table 5.

These results demonstrate that the yellow dye has one major impurity (designated impurity A) and that it is very homogeneous in composition. Because phthalic acid and phthalic anhydride do not separate well in this HPLC system, a maximum weight percent is given for the sum of both of the compounds. As was found with yellow dye, the major impurity in the green/yellow dye was impurity A. This dye was also very homogeneous in composition.

Impurity A appears to be an artifact produced during synthesis of the yellow dye because it is not present in the starting materials (phthalic anhydride and quinaldine) and is formed in similar quantities when QI is synthesized at ITRI. Analysis of impurity A by gas chromatography/mass spectrometry (after HPLC purification) indicated that its molecular weight is 395. This could possibly be a condensation of one molecule of quinaldine with two molecules of phthalic anhydride.

GENERATION OF AEROSOLS

Several generators, including a fluidized bed generator (FBG), a Trost jet mill generator and a Jet-O-Mizer, were evaluated as aerosol generators for dispersing smoke dyes in the inhalation study. Basically, an inhalation exposure system consists of an aerosol generator, a delivery system, and an exposure chamber. Figure 13 shows an exposure system constructed with an FBG in our chronic exposure laboratory. A 450-L Laskin exposure chamber was used to evaluate various aerosol generation systems (except for initial tests of a 2-inch FBG, which was in a laboratory setup) and also for the range-finding studies. Aerosol concentrations and particle size distributions in the chamber were monitored. Filter samples were taken every 5 to 10 min to determine the aerosol concentrations during a test run. Aerosol size distributions were determined by taking Lovelace multiple jet (LMJ) cascade impactor samples. We desired a system that would yield aerosols with mass median aerodynamic diameter (MMAD) between 3 and 5 microns (similar to stock material) and aerosol concentrations over 1 g/m^3.

Figure 11. High performance liquid chromatographic tracing showing impurities in yellow dye.

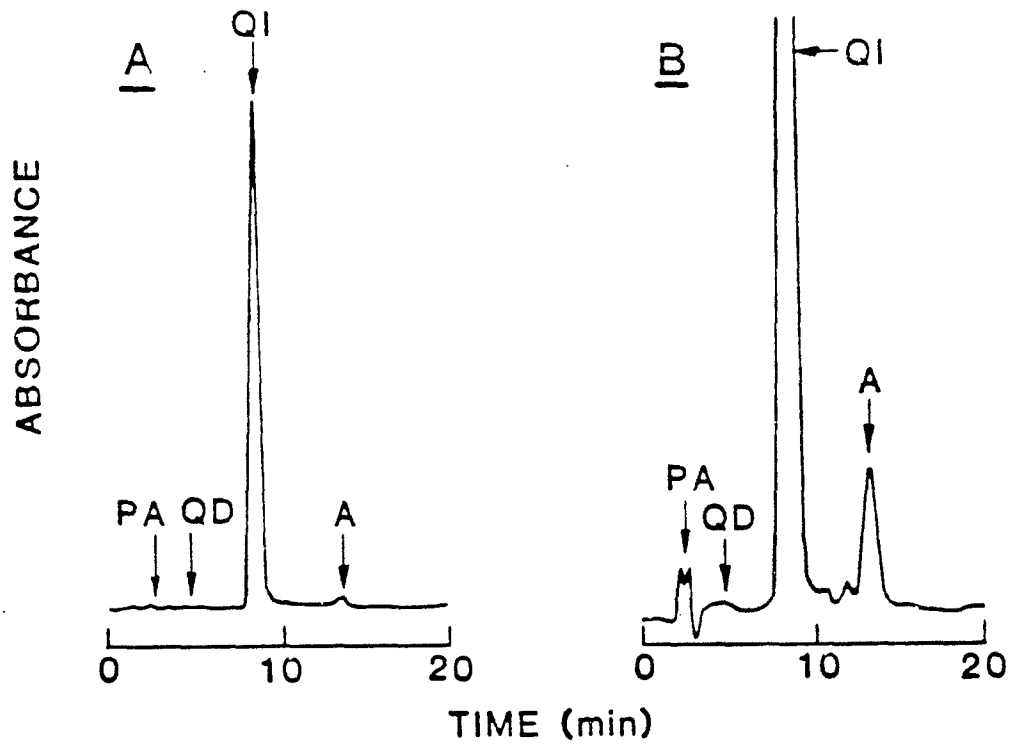


Chart A attenuation is 100 times that of chart B. QI, 2-(2'-quinolyl)-1,3-indandione; PA, phthalic acid or anhydride; QD, quinaldine; A, unknown.

Figure 12. Chart tracings from high performance liquid chromatograph analysis of green/yellow dye mixture.

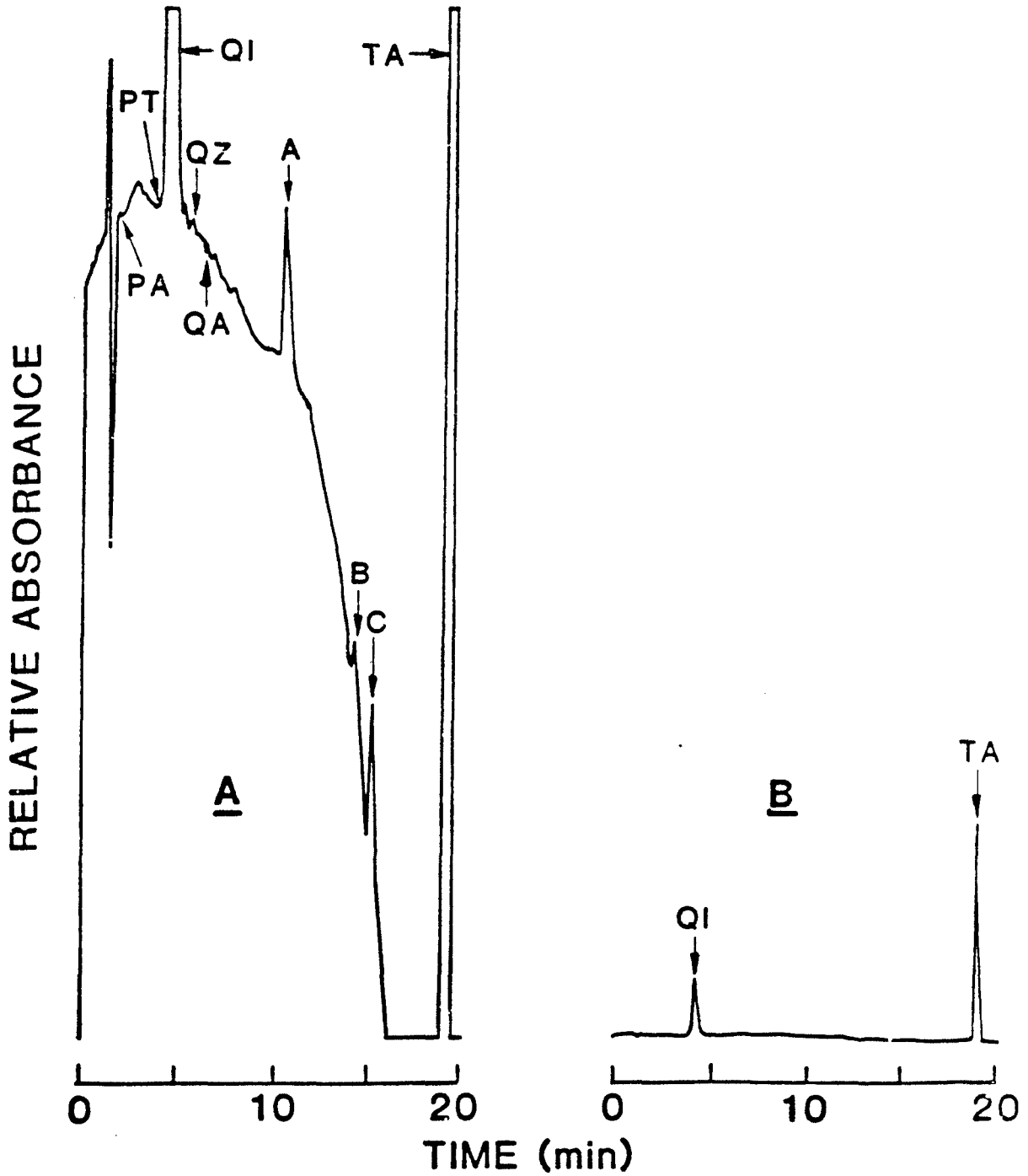


Chart B attenuation is 100 times that of Chart A. QI 2-(2'-quinclyl)-1,3-indandione; TA, 1,4-di-p-toluidinoanthraquinone; PT, p-toluidine; Qz, quinazarine; QA, quinaldine; PA, phthalic acid and anhydride.

Table 5. Amounts of Specific Impurities in Dyes

Dye	Impurities	Weight percent[a] (mean ± SD)[b]
Yellow	Phthalic anhydride and/or Phthalic acid	< 1.8 ± 0.1
	Quinaldine	< 0.4
	Impurity A	2.2 ± 0.1[c]
Yellow/Green Mixture	p-Toluidine	0.10 ± 0.03
	Impurity A	0.70 ± 0.04[d]
	Impurity B	0.09 ± 0.01[d]
	Impurity C	0.49 ± 0.02[d]
	Quinaldine	< 0.21
	Quinazarine	< 0.05

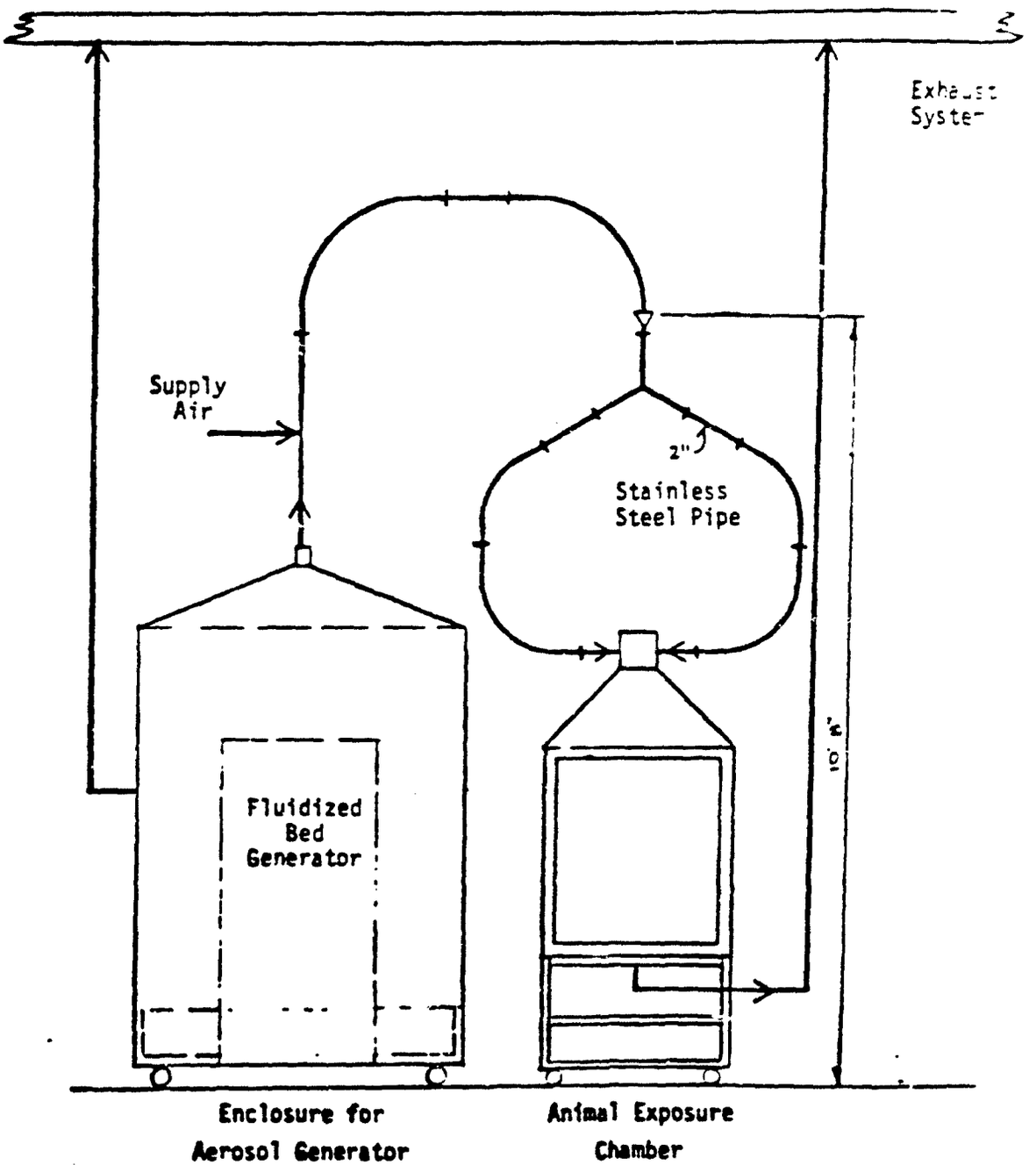
a. Weight of impurity ÷ weight of injected dye x 100 except where noted (see c & d).

b. n = 5

c. Area of A ÷ area of QI

d. Area of impurity peak ÷ sum of area of QI and TA x 100

Figure 13. Exposure system for acute toxicity range-finding exposures.



FLUIDIZED BED GENERATOR

In an FBG, the powder to be dispersed is mixed in a bed composed of inert particles. The bed is fluidized by passing air through it. The fine powder is suspended in the air, passing through it in a manner such that the resulting dust aerosol contains primarily single aerosol particles. We started with a 2-inch FBG to generate the SY dye (Figure 14). The material was mixed with 200 mL of stainless steel bed material. The flow rate through the FBG was 50 L/min under standard operation conditions. The aerosol concentrations produced in a chamber located directly above the generator (Figure 14) were determined for volume ratios (bed material: dye) of 10:1, 10:3 and 2:1 (Figure 15). The size distributions of these aerosols were measured with cascade impactors (Table 6). Clearly, the aerosol size was larger than the stock material and too big for our study. We then tried to deagglomerate SY dye with a Trost jet mill before using it in the FBG (Figure 16). The aerosol size distribution was only slightly smaller than the unmilled one (Table 6). Finally, a 4-inch FBG was tested in an exposure system (Figure 13) and produced an aerosol similar to that in the 2-inch FBG. Because the FBG was not satisfactory as an aerosol generator for the dye, we tested the Trost jet mill directly as a powder generator.

TROST JET MILL GENERATOR

The Trost jet mill (Garlock Inc., Newtown, PA) is used in food and chemical industries for grinding and classifying powders. We have adapted this device as an aerosol generator for inhalation toxicology studies. The generation system includes a feeder, a jet mill, and a noise reducer. The jet mill (Figure 17) consists of a shallow round channel and two opposing high-pressure jets that issue through orifices spaced around the periphery of the channel. Powder is carried into the mill by jets operated at high velocities. Air circulating at high speed in the channel creates turbulence and centrifugal forces. The rotating gas is discharged at the center of the chamber, carrying fine particles with it, while more coarse particles are centrifuged for recirculation. In the initial test, the dye powder was divided into equal amounts in a flat pan and manually fed into the Trost jet mill by aspiration. A muffler stuffed with sound absorbing foam was used to reduce the high-frequency noise (about 100 db) caused by jets to 70 db. A Trost jet mill with two opposing jets operated at 90 and 60 psig had a flow rate of about 6 ft^3/min.

Two 1-hour test runs were performed to evaluate the Trost jet mill as a powder generator. With SY dye, the manual feed rate was 1.05 g/min. The mean aerosol concentration and SD over 39 filter samples taken during the test run were 901 ± 112 mg/m^3. The MMAD obtained by three impactor samples was 3.8 ± 0.5 μm with a geometric standard deviation (σ_g) of 2.0 ± 0.1. The initial test for SG/SY dye mixture lasted only 30 min before the jet became clogged. It was found that the dye mixture was much more sticky than the SY dye, causing clogging inside the jet. We decided to modify the air jet inside

Figure 14. Two-inch fluidized bed generator used to produce aerosols in the laboratory.

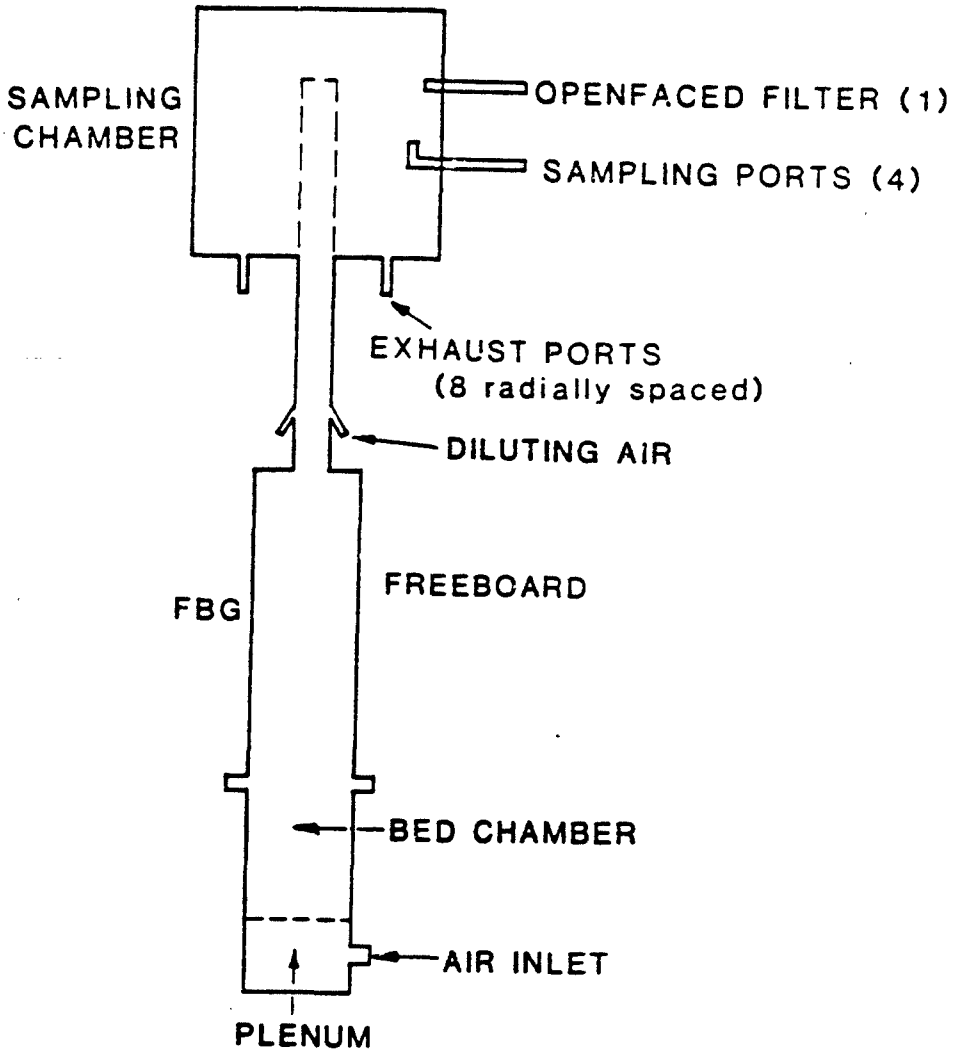


Figure 15. SY dye (unmilled material) aerosol concentration generated from 2-inch FBG.

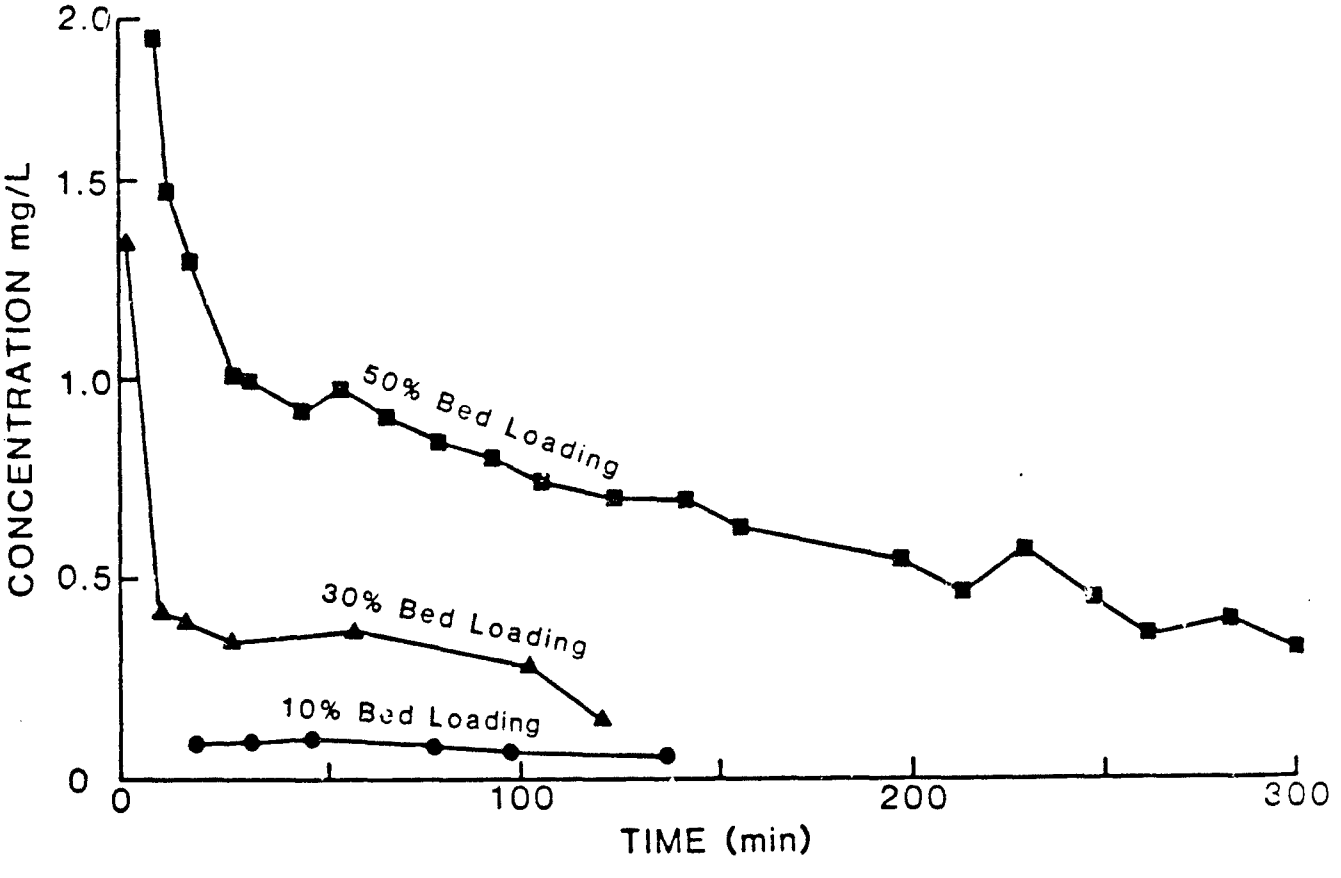


Table 6. Effect of Bed Loading and of Milling on the Measured Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (σ_g) of SY Aerosolized by an FBG[a]

	MMAD in μm (σ_g)		
FBG Bed Loading[b]	1 hr	2 hr	3 hr
10:1	19.6 (1.80)	22.0 (2.26)	
10:3	8.8 (2.00)	9.71 (1.85)	
2:1	8.7 (2.26)	6.04 (2.16)	7.94 (1.96)
(SY from Trost jet mill 10:3)	7.5 (2.30)	6.4 (2.1)	6.7 (2.72)

a. Samples taken at 1, 2, or 3 hr after initiation of fluidized bed generation of particles.

b. Flow rate is 50 L/min through the FBG.

Figure 16. SY dye aerosol concentration generated from FBG. Trost-milled 5 times; 30% bed loading.

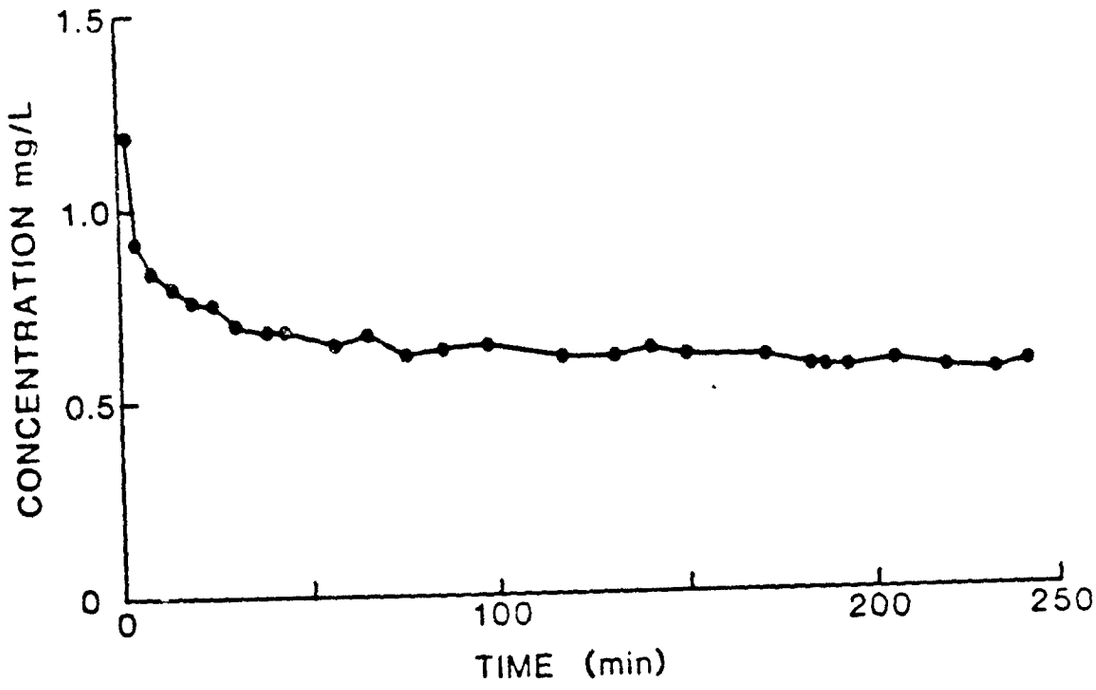
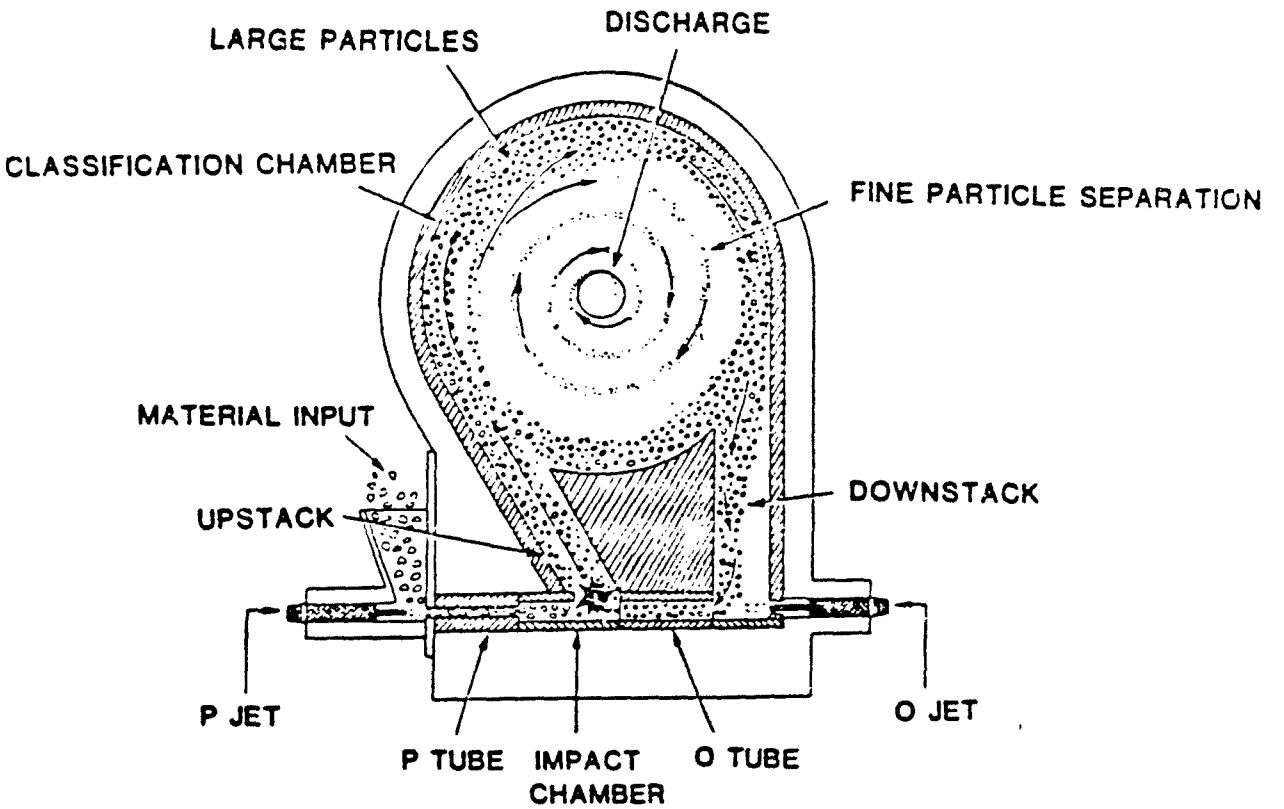


Figure 17. Schematic of a Trost jet mill.



the Trost jet mill to allow greater clearance. With the modification, we were able to generate the dye mixture for a 1-hour exposure. The manual feed rate was 0.92 g/min. The mean aerosol concentration and SD over 41 filter samples was 680 ± 130 mg/m^3. The measured MMAD from three impactor samples was 3.3 ± 0.2 μm, and σ_g was 1.8 ± 0.2. After the exposure, the Trost jet mill was opened for cleanup. A heavy deposit of the SY/SG dye mixture was found in the jets, and the mill was partially plugged after 1 hr continuous operation.

JET-O-MIZER

The Trost jet mill appeared inadequate to generate dyes for continuous long-term operation because of the dye deposit clogging the inside of the mill, so an alternative generator was tested. A Jet-O-Mizer (Figure 18) was obtained from Fluid Energy Corp., Hatfield, PA. In principle and construction, it is very similar to the Trost jet mill, but it is three times the size of the Trost mill and has higher flow rates (14 ft^3/min operated at 43 psig). The generation system consisted of a powder feeder, a Jet-O-Mizer, a one-stage impactor (right angle turn in line), and a noise reducer (Figure 5). A dry chemical feeder with digital speed control (Model 302, AccuRate, WI) was used as a powder feeder. A T-section between the exit of the Jet-O-Mizer and the silencer acted as a single stage impactor, with larger particles unable to make the 90° turn and being collected in the collection bin. By varying the size of the incoming tube, we could change the effective cutoff diameter of the aerosol (ECD_{50}):

$$ECD_{50} = 1.23 \times 10^4 \sqrt{\frac{\eta d^3}{F}}$$

where d is the diameter of the tube (cm), F is the flow rate (cc/sec) and η is the viscosity of the air (poise). With a tube diameter of 5/8 inch and a flow rate of 14 ft^3/min, the ECD_{50} was 4.1 μm.

Aerosol concentration in this system is determined mainly by the feed rate and the air flow rate through the Jet-O-Mizer. Feed rate is controlled by the screw's profile and its rotational speed. Initial tests indicated that both dyes tended to stick to the surface of the screw, causing the feed rate to be lower than the specified rate and the output to be unstable. A vibrator was attached to the delivery tube of the screw feeder to keep the powder loose. This prevented the powder from sticking to the screw and allowed it to flow freely and continuously. Calibration of the screw feeder was done for both SY and SY/SG dyes using a 1/2-inch helix screw (5 turns per inch) with center rod and with the vibrator attached (Figure 19). The feed rate was linear over a range from 0.05 to 2 g/min and was independent of the material used. For SY dye, a larger screw (3/4 inch without center rod, 2 turns per inch) was also tested; the calibration curve is shown in Figure 20. Variation of feed rate in a 1-hour test was usually about 5 percent when the feed rate was higher than 0.5 g/min (Figure 21).

Figure 18. Schematic of a Jet-O-Mizer.

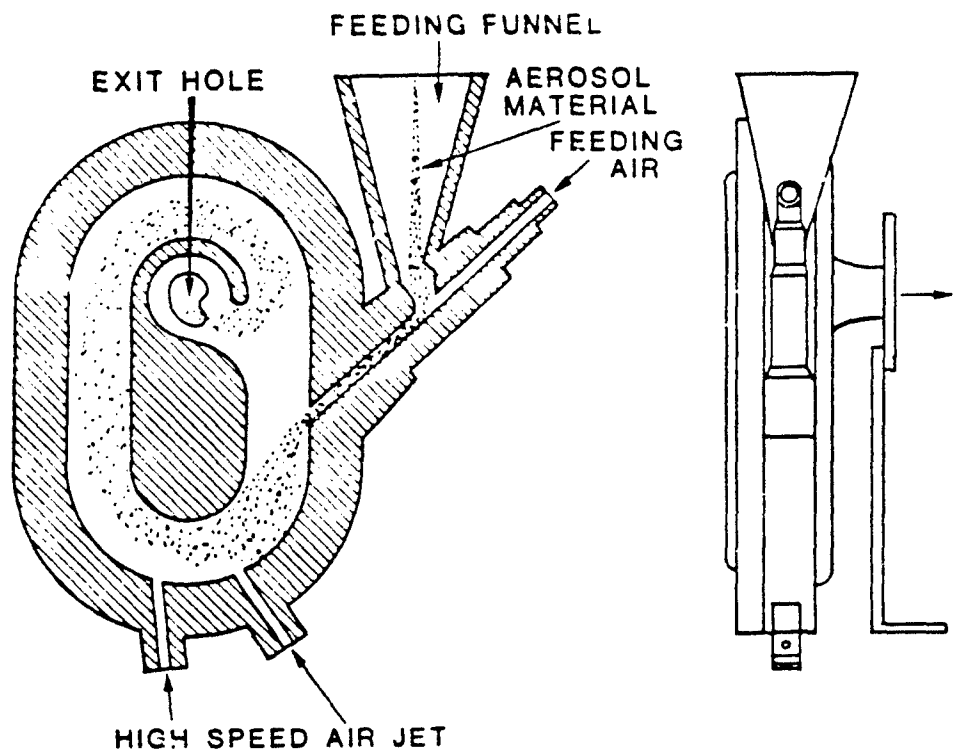


Figure 19. Calibration curve of the feed rate from a screw feeder using a 1/2-inch screw, 5 turns per inch with center rod. The circles are mean feed rate measured in 1-hr test run for SY; squares are the dye mixture. The straight line is the linear regression line.

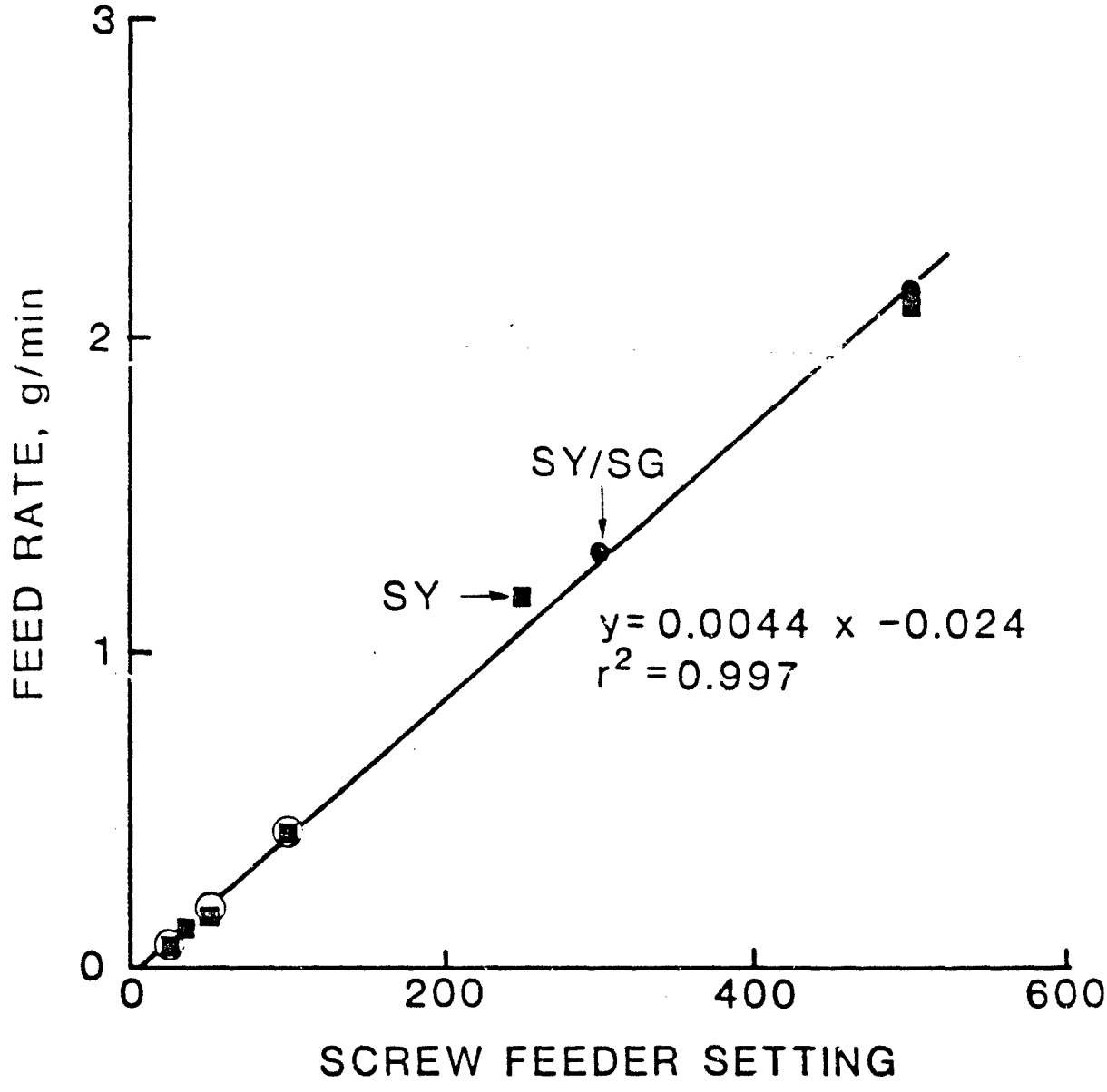


Figure 20. Calibration curve of the feed rate from a screw feeder using a 3/4-inch screw without center rod (2 turns per inch). The circles are the SY mean feed rate measured in 1-hr test run; the straight line is the linear regression line.

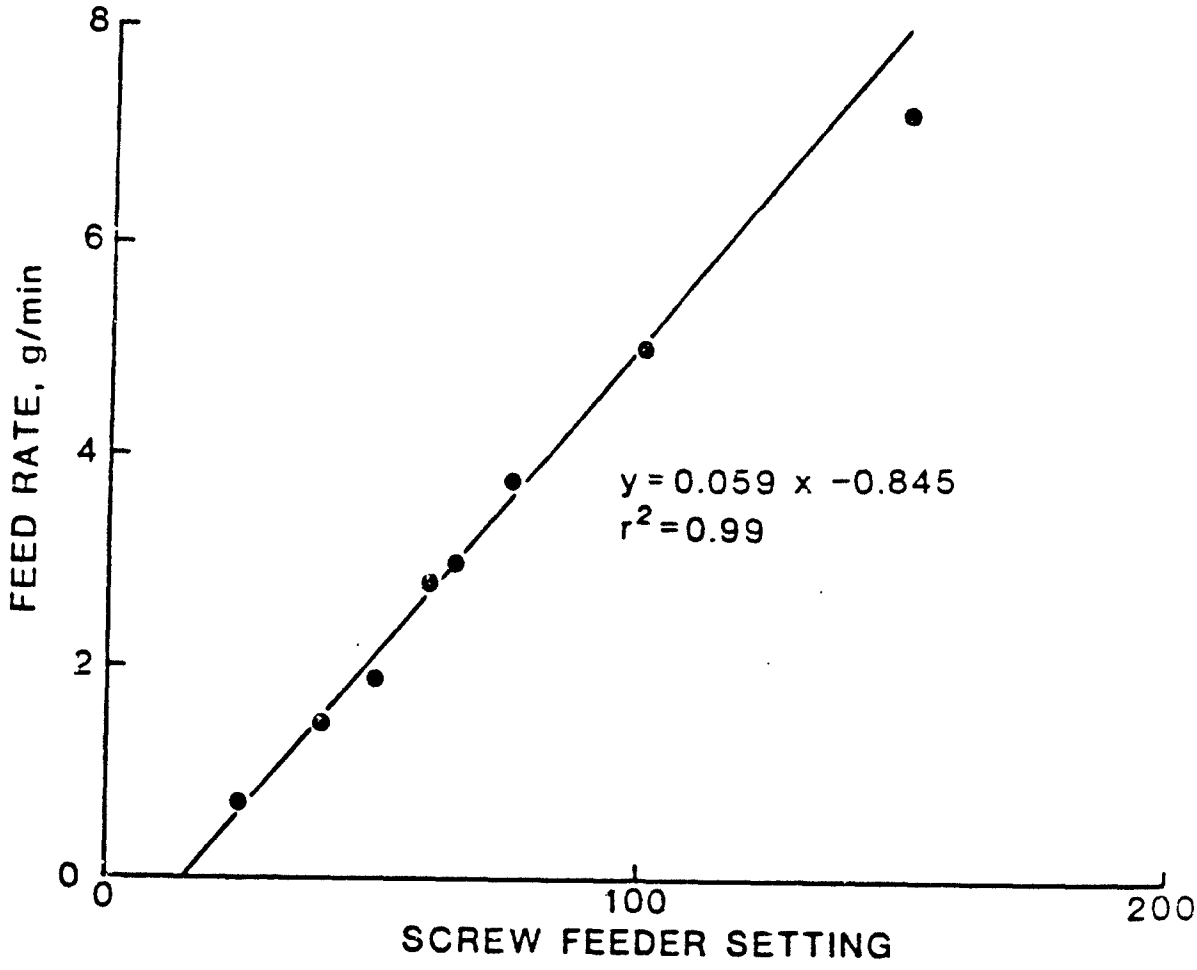
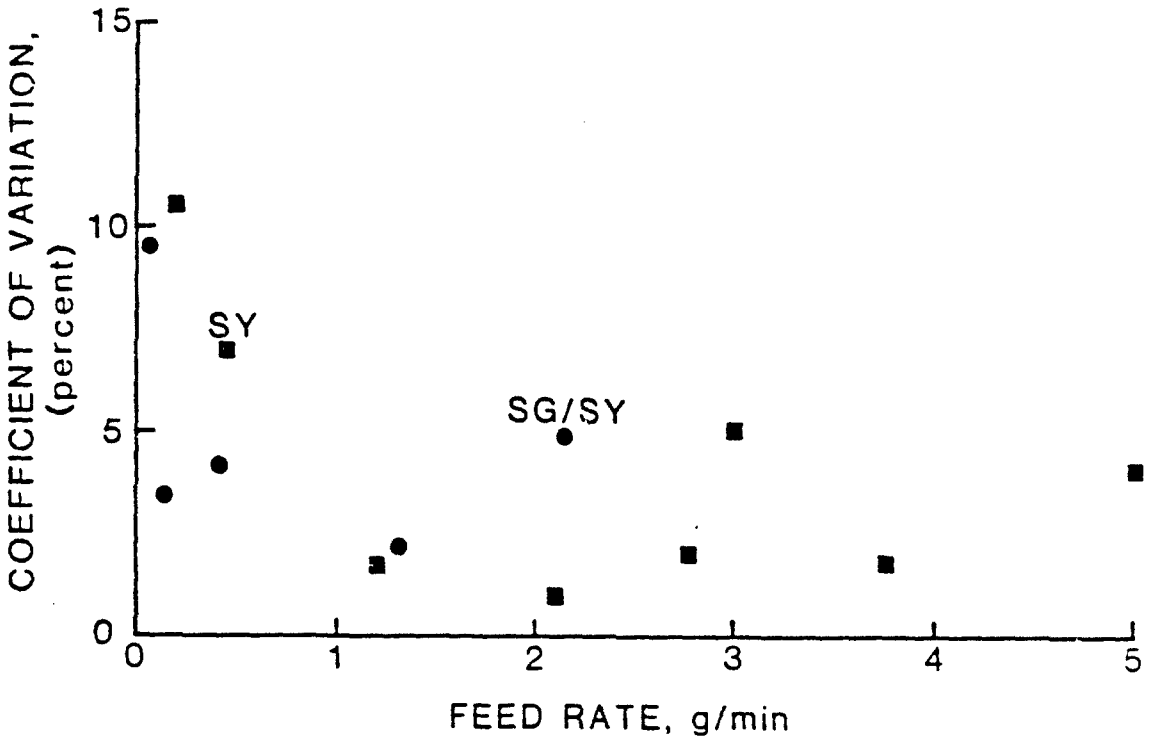


Figure 21. Coefficient of variation in the feed rate over 1-hr test run.



Aerosol concentrations measured in the exposure chamber are shown in Figure 22. A minimum concentration of 40 mg/m^3 was obtained for both dyes at a feed rate of 0.06 g/min. As the feed rate increased, however, the aerosol concentration of SY dye was higher than the SG/SY mixture because the aerosol of the mixture generated from the Jet-O-Mizer was larger and more was removed in the one-stage impactor than the SY dye aerosol. For SY dye, a concentration above 1200 mg/m^3 was achieved at a 3 g/min feed rate (Figure 22). Variation in the measured concentration during a 1-hour exposure was generally about 5 to 10 percent (Tables 7 and 8). The aerosol particle size measured by the cascade impactor is shown in Figure 23. There appeared to be a slight concentration effect. The magnitude and importance of this effect was analyzed by simple linear regression. The slope of the line is 0.0019, which differs from zero ($p = 0.0002$). The MMAD ranged from 3 μm at 50 mg/m^3 to above 5 μm at concentrations higher than 500 mg/m^3. The σ_g were about 2. Stability of 6-hour exposures for both SY and SG/SY at concentrations above 1 g/m^3 were about 15 percent (Figure 24). No cleanup of the Jet-O-Mizer was required during this continuous run. However, at the end of the 6-hour exposure, some deposit in the mill was found, and this had to be removed for further operation.

CHAMBER HOMOGENEITY STUDIES

The purpose of the homogeneity study was to determine the distribution of solvent yellow (SY) aerosol in the Hazleton 2000 (HC 2000) animal exposure chambers that are to be used in the subacute and subchronic inhalation toxicity studies. Prior evaluation of aerosol distribution in this chamber in our laboratory has shown a side-dependent variation of about 10 percent in aerosol concentration in tests using an aerosol of about 2.0 μm aerodynamic diameter and very low aerosol concentration (Griffis et al., Fund. Appl. Toxicol 1: 8-12 1981). Because this report indicated that an animal's location within the chamber had a slight effect on the lung burden of inhaled aerosol, animals are usually rotated among the tiers of the chamber during long-term inhalation exposures in our laboratory. In the present study, we evaluated the distribution of SY aerosol in the HC 2000 chambers using the aerosol generation system as described in ITRI Protocol FY-84-009 and at both a high (approximately 250 mg/m^3) and a low (< 50 mg/m^3) concentration of aerosol.

The HC 2000 chamber was tested while fully loaded with cages and cage trays at all tiers. Sampling ports with 1/4-inch sampling probes were positioned on the door of the exposure chamber, as illustrated in Figure 25. One probe was placed above each of the six tiers of animal cages, and a reference probe was placed at the middle of the chamber (Figure 25). The reference probe remained at a constant position in the middle of the chamber. The other six probes were moved to sample at three planes in the chamber: front plane (at 0.25 of the distance into the chamber); middle plane (at 0.5 of the distance into the chamber); and back plane (at 0.75 of the distance into the chamber).

Figure 22. Mean aerosol concentration measured in a 27-inch chamber for SY and SY/SG mixture.

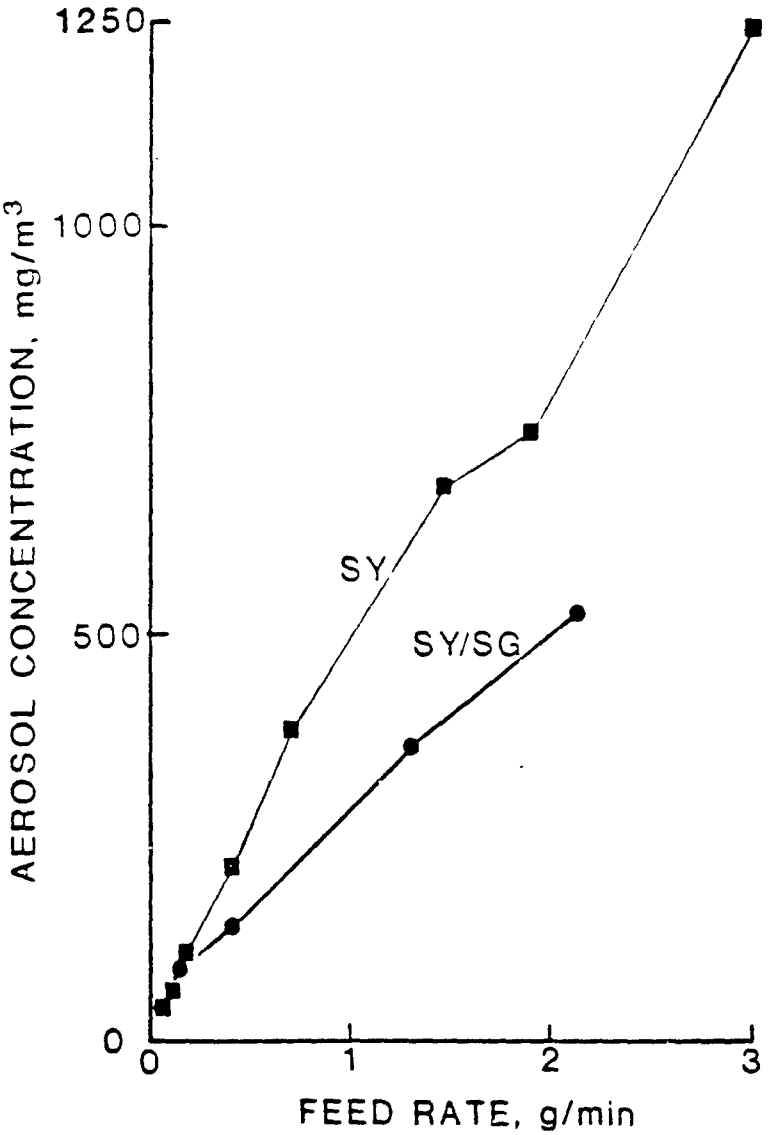


Table 7. Effect of Feed Rate on Chamber Concentration of Yellow Dye

Feeder: 1/2": helix, 5 turns/inch, with center rod; vibrator on nozzle

Feeder Setting	Feed Rate (g/min)	Chamber Concentration $\bar{X} \pm SD$ (mg/m^3)	MMAD (μm)	σ_g
025	0.064 (0.064 ± 0.005)	41 ± 2	3.8	2.1
050	0.20 (0.18 ± 0.02)	107 ± 6	3.1	2.0
100	0.45 (0.42 ± 0.03)	213 ± 20	3.3	2.0
250	1.20 (1.17 ± 0.02)	398 ± 34	3.5	2.1

Table 8. Effect of Feed Rate on Chamber Concentration of Green/Yellow Dye Mixture

Feeder: 1/2": helix, 5 turns/inch, with center rod; vibrator on nozzle

Feeder Setting	Feed Rate X ± SD (g/min)	Chamber Concentration X ± SD (mg/m^3)	MMAD (μm)	σ_g
25	0.066 ± 0.006	38.6 ± 4.5	3.8	1.9
50	0.176 ± 0.006	88 ± 8	3.8	1.9
100	0.42 ± 0.018	139 ± 9	3.3	2.1
300	1.31 ± 0.029	358 ± 31	4.4	2.2
500	2.14 ± 0.106	524 ± 25	4.4	2.2

Figure 23. Measured mass median aerodynamic diameter (MMAD) of SY and SY/SG dyes as a function of aerosol concentration measured by a Lovelace Multijet Impactor.

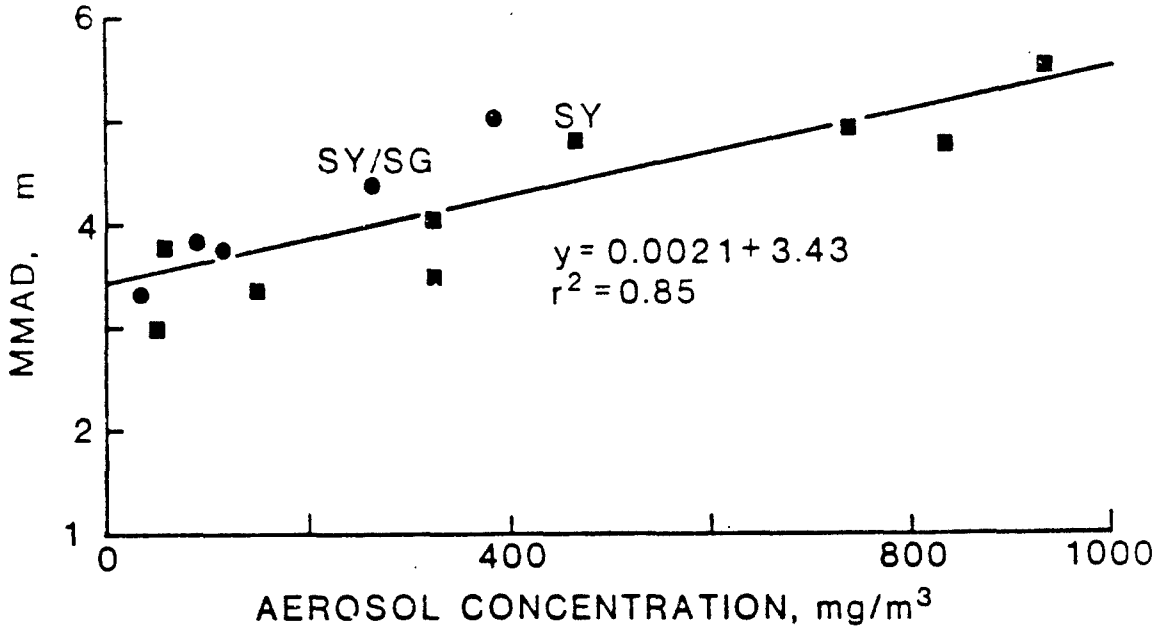


Figure 24. Stability of solvent yellow and solvent yellow/solvent green aerosol concentration during a 6-hr exposure.

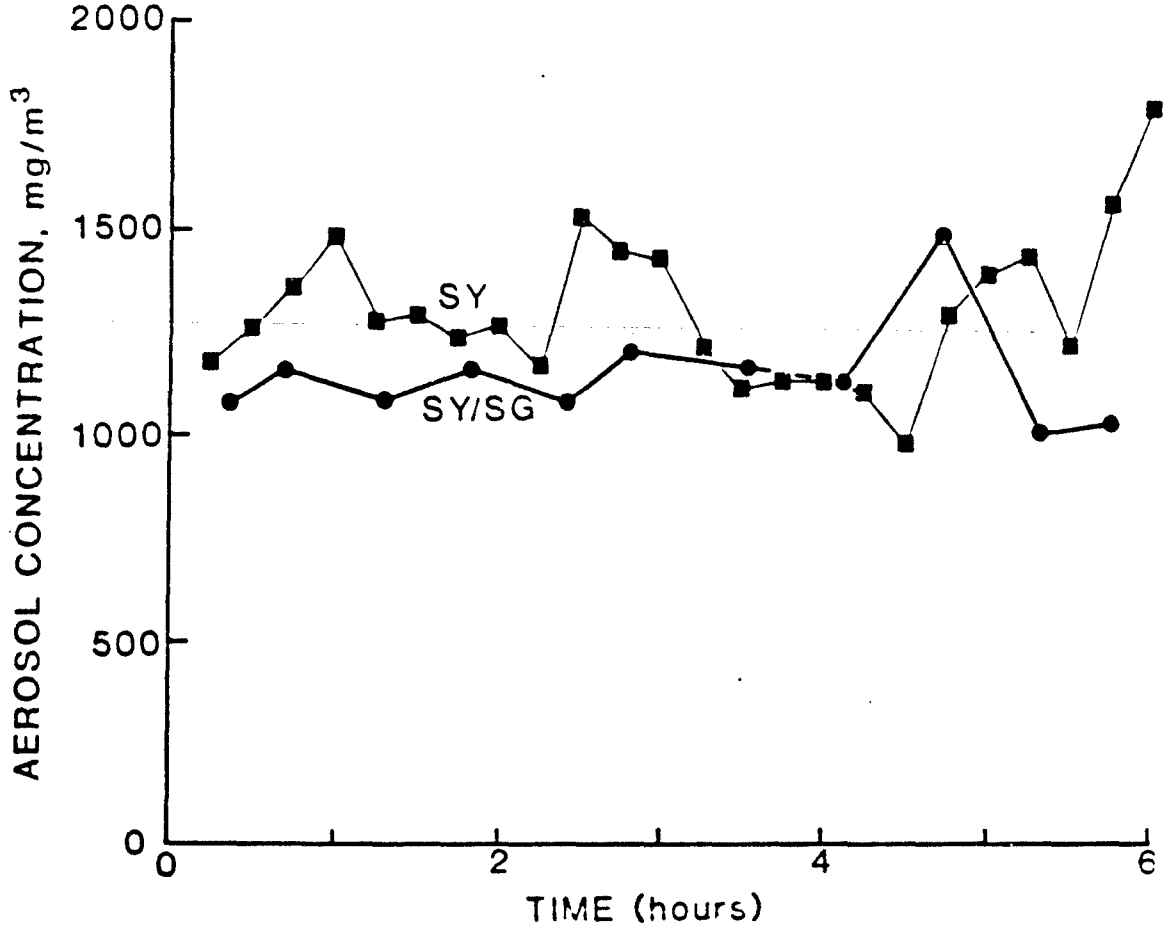
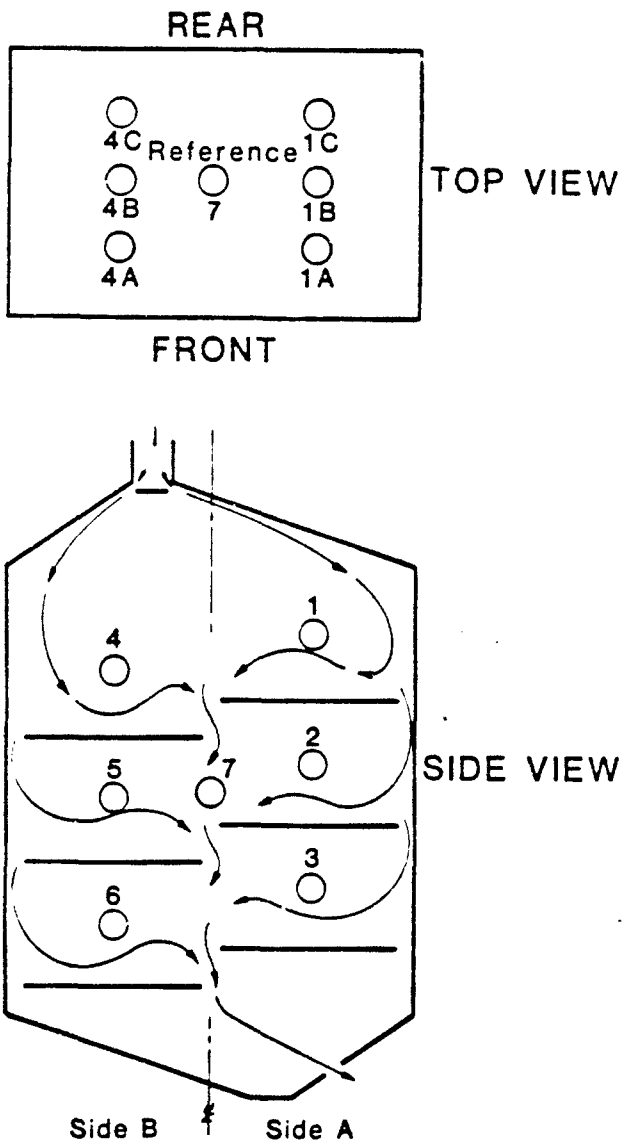


Figure 25. Schematic of a Hazleton 2000 chamber.



Vacuum was used to pull air samples through the probes to an online filter followed by a critical orifice. Sampling flow rates were approximately 1 L/min. Critical orifices for each probe were calibrated individually with bubbler flow meters. The mean of ten calibration runs was used to determine the flow rate for each individual probe.

Seven filter samples were taken simultaneously. These were the six points in one randomly chosen plane of the chamber as specified in the random sample design and one at the reference point. Triplicate samples were taken at each sampling point and at the reference point (resulting in a total of nine replicates at the reference point during the sampling of the three planes). The time required to take a sample is dependent on the aerosol concentration. In this study, sampling time was 4 min for the high concentration and 40 min for the low concentration. The sampling rate of approximately 1 L/min at each of seven sampling probes represented less than 2 percent of the total chamber flow of 420 L/min.

Aerosol concentration was determined from the weight of material collected on the filter, the flow rate at each probe, and the sampling time. The aerosol concentration at each sampling point was divided by the concentration of aerosol at the reference point in the samples taken simultaneously. These concentration ratios were analyzed as a three-factorial design using Biomedical Data Processing (BDMP) and Statistical Analysis System Procedure (SAS PROC) ANOVA computer programs. An overall coefficient of variation of 20 percent or less is considered acceptable.

The concentrations of the aerosol generated in the study were 23.6 ± 4.4 mg/m^3 (low concentration) and 265 ± 21 mg/m^3 (high concentration) as determined by nine samples taken at Reference Point #7 (Table 9). There was a concentration effect on the aerosol size, with the higher concentration of aerosol having a larger MMAD. Results of the homogeneity study are shown in Table 10. There was greater variability in the measurements taken at the higher concentration. ANOVA analyses for both the low and the high aerosol concentrations showed a statistically significant ($P < 0.05$) variation in aerosol concentrations within the chamber (Tables 11 and 12). At the low concentration, these differences were significant with regard to all three parameters studied: height in the chamber, side of the chamber (width), and plane of the chamber (depth). At the high concentration, only the height and side in the chamber were determining factors in the aerosol concentration.

A Scheffe multiple comparisons procedure was done to find where the differences lie in each of the three factors (Tables 13 and 14). At both concentrations, Side B aerosol concentrations were higher than those of Side A. This agrees with the previous evaluation of this chamber using a smaller aerosol at a very low concentration. It is also evident in the present study that the bottom tiers (3 and 6) had lower concentrations of aerosol than did the higher tiers (1, 2, 4, 5). These differences were greater at the higher concentration of dye aerosol than the lower. This is to be expected for the larger particle size of the higher concentration because larger particles would not be expected to negotiate turns in the chamber as well as small particles.

Table 9. Aerosol Characterization for Chamber Homogeneity Study

Mean Concentration[a] (mg/m^3)	Standard Deviation	Coefficient of Variation	MMAD (μm)	σ_g
23.6	± 4.4	19%	3.3	1.9
265	± 20.6	8%	4.7	1.9

a. This is the mean of 9 samples taken at Position 7 in the chamber. See Figure 1 for description of chamber positions.

Table 10. Homogeneity Study[a]: Ratio of Aerosol Concentration at Individual Sampling Points to Concentration at Reference Point

High Concentration (265 ± 21 mg/m^3)

	Tier					
	1	2	3	4	5	6
Front (A)	0.99 ± 0.02	0.91 ± 0.03	0.87 ± 0.13	1.16 ± 0.05	1.11 ± 0.04	1.06 ± 0.06
Center (B)	1.12 ± 0.13	0.84 ± 0.28	1.01 ± 0.17	1.11 ± 0.13	1.04 ± 0.08	0.85 ± 0.23
Back (C)	1.06 ± 0.17	0.97 ± 0.06	0.75 ± 0.12	1.12 ± 0.20	1.09 ± 0.12	1.14 ± 0.16

Low Concentration (23.6 ± 4.4 mg/m^3)

	Tier					
	1	2	3	4	5	6
Front (A)	0.90 ± 0.11	0.89 ± 0.08	0.79 ± 0.06	1.00 ± 0.10	0.96 ± 0.01	0.95 ± 0.09
Center (B)	0.98 ± 0.02	0.95 ± 0.04	0.84 ± 0.05	0.96 ± 0.07	0.96 ± 0.02	0.87 ± 0.13
Back (C)	1.02 ± 0.06	1.02 ± 0.09	0.98 ± 0.07	1.09 ± 0.06	0.97 ± 0.05	1.11 ± 0.08

a. Values are $\bar{X}$ ± SD, n = 3. The number represents the ratio of the concentration of dye at any one point to the concentration at the reference point in simultaneously taken samples. See Figure 1 for explanation of sampling point locations.

Table 11. Anova for Low Concentration Dye Study

Overall Mean of Concentration Ratios	Coefficient of Variation	Overall F Value	P[b]
0.96	8%	7.8	0.0001

Analysis by Parameter:

Parameter	DF[a]	Sum of Squares	F Value	P
Depth	2	0.147	12.6	0.0001
Width	1	0.042	7.2	0.0101
Height	2	0.039	3.3	0.0456

Primary Statistics:

Source	DF	Sum of Squares	Mean Square
Model	5	0.228	0.046
Error	48	0.281	0.006
Corrected Total	53	0.509	

a. DF = degrees of freedom

b. P = probability

Table 12. Anova for High Concentration Dye Study

Overall Mean of Concentration Ratios	Coefficient of Variation	Overall F Value	P[b]
1.01	15%	9.31	0.0001

Analysis by Parameter:

Parameter	DF[a]	Sum of Squares	F Value	P
Depth	2	0.004	0.08	0.921
Width	1	0.212	9.38	0.004
Height	2	0.223	4.95	0.011

Primary Statistics:

Source	DF	Sum of Squares	Mean Square
Model	5	0.439	0.088
Error	48	1.083	0.023
Corrected Total	53	1.522	

a. DF = degrees of freedom

b. P = probability

Table 3. Scheffe Multiple Comparisons for Low Concentration Study

Scheffe

Grouping of Means[a,b]	Mean	Depth
A	1.03	Back
B	0.93	Center
B	0.92	Front

Scheffe Grouping of Means	Mean	Height
A	0.99	Top
A,B	0.96	Middle
B	0.92	Bottom

Scheffe Grouping of Means	Mean	Width
A	0.99	Side B
B	0.93	Side A

a. P = 0.05; DF = 48; MSE = 0.006
Critical value of F = 3.19
Minimum significant difference = 0.06

b. Means with the same letter are not significantly different.

Table 14. Scheffe Multiple Comparisons for High Concentration Study

Scheffe Grouping of Means[a,b]	Mean	Depth
A	1.02	Back
A	1.00	Center
A	1.02	Front

Scheffe Grouping of Means	Mean	Height
A	1.10	Top
A	0.99	Middle
B	0.95	Bottom

Scheffe Grouping of Means	Mean	Width
A	1.08	Side B
B	0.95	Side A

a. P = 0.05; DF = 48; MSE = 0.022
Critical value of F = 3.19
Minimum significant difference = 0.08

b. Means with the same letter are not significantly different.

The inhomogeneities observed, while statistically significant, can be expected to have minimal effects on the lung burdens of the exposed animals. The range in means for each type of sampling positions (back, center front, top, middle, bottom, Side A, or Side B) is only from 0.92 to 1.10 (Tables 13 and 14).

All chamber exposure systems were run for 6 hours as a final check of the stability of the aerosol generation system. Results are shown in Table 15. For the particle size analysis, data from the low and high exposure levels used in the homogeneity study have been averaged with the data from the high and low exposure levels of the trial run.

Table 15. Aerosol Concentrations and Particle Size in 6-hr Test Runs

Concentration[a] (mg/m^3)	Coefficient of Variation (%)	MMAD (μm)	σ_g
16 ± 2, n = 9	13	3.0 ± 0.4[b]	2.0
57 ± 11, n = 6	19	4.0	2.0
248 ± 29, n = 9	12	4.6 ± 0.1[b]	2.0

a. Samples taken at evenly spaced intervals during 6-hour period.

b. Mean ± SD of two samples, one taken during homogeneity study, and one taken during 6-hour.

GLOSSARY

Green component of yellow/green dye: green dye, solvent green, SG

Major compound in yellow dye: 2-(2'-quinolyl) - 1,3-indandione, QI

Major compound in green dye: 1,4-di-p-toluidinoanthraquinone, TA

Stock yellow dye: yellow dye, solvent yellow, SY

Stock yellow/green dye; yellow/green dye mix, SY/SG

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